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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
ALAN K. SMITH, ET AL.	: EXAMINER: BELYAVSKI
SERIAL NO: 10/668,214	:
FILED: SEPTEMBER 24, 2003	: GROUP ART UNIT: 1644
FOR: HUMAN LINEAGE COMMITTED CELL COMPOSITION WITH ENHANCED PROLIFERATIVE POTENTIAL, BIOLOGICAL EFFECTOR FUNCTION, OR BOTH; METHODS FOR OBTAINING SAME; AND THEIR USES	:

DECLARATION UNDER 37 C.F.R §1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I Douglas M. Smith state that:

I am a named inventor of this application.

I understand that the U.S. Patent Office has rejected the claims of this application in view of U.S. patent no. 5,858,358 (U.S. '358) and Stacey et al (Manual of Cell Culture Techniques, 1990, pages 1-63) as a basis to allege that the claims of the above-identified application would have been obvious to one of skill in this field.

I also understand that the U.S. Patent Office has rejected the claims of this application on the basis that the application does not enable the use of cultured cells as defined in the claims for immunotherapy.

I disagree with both points and elaborate on the reasons below.

I. The use of the cultured T-cells for Immunotherapy is enabled by what is described in the patent application

The examiner states that it is unpredictable how to correlate *in vitro* results with *in vivo* use. However, the National Institutes of Health specifically use cytokine release as a potency assay for therapeutic T-cells in their Phase II clinical trials for T-cell based immunotherapy of melanoma as mandated by the Food and Drug Administration (FDA). (Dudley et al (2002) *Science 298*:850-854; and Dudley et al (2005) *J. Clin. Oncol.* 23(10): 2346-57).

A specific demonstration of enhanced cytokine release is provided in the data below comparing tumor infiltrating lymphocytes (TILs) expanded under perfusion conditions as defined in the claims to TILs produced from the same donor under conventional hemi-depletion conditions conducted by direct head-to-head comparison. This experiment utilizes the exact potency assay using identical methodology accepted subsequently by the FDA (for early phase I and II trials) (see Dudley et al (2002) *Science 298*:850-854; and Dudley et al (2005) *J. Clin. Oncol.* 23(10): 2346-57).

Expansion of melanoma reactive tumor infiltrating lymphocytes (TILs) under perfusion culture conditions compared to hemi-depletion conditions in tissue culture flasks.

Well-characterized cryopreserved/thawed MART-1 reactive TILs from an HLA-$A2^+$ melanoma patient were expanded in a clinical-scale bioreactor system using preferred methodology as described in the claims for the invention (For an overview of the perfusion bioreactor system see Mandalam et al. Chapter 13 in: Schindhelm K, Nordon R eds. Ex Vivo Cell Therapy: Academic Press, San Diego, 1999: 273-291). Bioreactor cassettes (850 cm^2 PETG tissue culture-treated growth surface) were inoculated at 20 x 10^6 TILs per cassette (6.67 x 10^4 cells/ml) (A) or 5 x 10^6 TILs per cassette (1.67 x 10^4 cells/ml) (B) together with 1 x 10^9 irradiated allogeneic feeder cells (peripheral blood mononuclear cells) in complete medium containing anti-CD3 mAb (OKT3, Orthoclone, 30 ng/mL) and 6000 international

units (IU) per ml recombinant human interleukin-2 (rhIL-2) (Chiron Corp., Emeryville, CA). Complete medium (CM) consisted of RPMI 1640 and AIM V (1:1 ratio), 25 mM HEPES, 50 U/mL penicillin, 50 mcg/mL streptomycin, 20 mcM 2-mercaptoethanol, and 5% human AB serum (Valley Biomedical Products and Services, Inc., Winchester, VA). The rate of continuous perfusion for medium containing IL-2 alone was adjusted to maintain a lactate tolerance level of 0.5 - 1.0 mg/ml as determined by sampling and measurement in the waste medium. Small-scale PETG (polyethylene terapthalate-glycol plastic) T-flasks (InVitro Scientific Products, Inc., Ventura, CA) as controls for each bioreactor were established at identical inoculum densities per unit surface area and maintained under conditions mimicking as closely as possible the conditions of the clinical-scale system with respect to medium height (3 mm), gas (20% oxygen environment), and medium exchange rates. A standard low density process for expansion of T-cells (< 1 x 10^6 TILs/ml as maximum T-cell density) in polystyrene T-flasks with manual medium replacement and hemi-depletion steps on days 2, 5, 7, 9 and 12 was included for comparison as an additional control (Riddell and Greenberg, 1990 as adapted by Dudley et al, 2002 and 2005). In contrast to the standard hemi-depletion control group as specified by Riddell, Greenberg and Dudley et al., bioreactors and PETG T-flasks were maintained by perfusing or manually exchanging culture medium based on lactate measurements with subsequent T-cell expansion to high cell density (>19 x 10^6 T-cells/ml) in a continuous process without subculture. For the control process in 25 cm^2 PETG T-flasks, a constant medium volume of 7.5 mls providing a 3 mm liquid height was maintained throughout the entire culture period. Alternatively, a 75 cm^2 PETG T-flask using 22.5 mls medium (also providing a 3 mm medium height) may be used. This medium depth provides conditions for gas exchange using a 20% oxygen enviroment which are comparable to the clinical scale system. In contrast to the continuous replacement of medium in the clinical-scale bioreactor system, medium exchange in the PETG T-flasks was carried out manually as

a single replacement at a rate of at least 25-100% per day based on lactate production as described in the claims. In addition, T-cells removed in the spent medium were recovered by centrifugation, resuspended in fresh medium and returned to the PETG T-flasks thus ensuring that no cells were lost from the culture during medium exchange. This latter step was not required at clinical-scale as <1% of T-cells are removed in the bioreactor waste medium as achieved in the bioreactor design. Thus, the T-cell density was not reduced or adjusted at any time during the culture process while maintaining a constant culture volume with frequent medium replacement during progressive T-cell expansion from low to high density.

The cultures were harvested for analysis in assays for cytokine secretion (ELISA) on day 14. The viability of all cultures was >95% as assessed by hemacytometer count using trypan blue dye exclusion or by flow cytometry using 7-AAD dye exclusion. Cytokine secretion was assessed as described in Dudley et al (2002 and 2005) and the legend of Figure 1. Briefly, 10^5 TILs were incubated in round-bottom microwells (0.2 mls) pre-coated with plastic–immobilized OKT3 mAb. Alternatively, 10^5 TILs were co-cultured with 10^5 irradiated T2 or melanoma tumor cells as indicated in Figure 1. Supernatants were harvested after a culture period of 24 hours and cytokine concentration was determined by ELISA.

As shown in Figure 1-A, GM-CSF and IFN gamma cytokine release in response to immobilized anti-CD3 mAb (polyclonal T-cell receptor triggering) was enhanced under perfusion culture conditions in both small-scale PETG control T-flasks or clinical-scale bioreactor systems when compared to the standard low density hemi-depletion T-flask controls (Figure 1-A). A similar pattern of high antigen-specific IFNgamma release in response to MART-1 peptide loaded T2 cells or against a panel of MART-1-expressing HLA-A2(+)ve melanoma cells (Mel526, TC624) was observed (Figure 1-B). Again, the greatest levels of tumor antigen-specific cytokine release were demonstrated for TILs expanded at high density under perfusion culture conditions compared to TILs produced in

standard low density control T-flasks maintained by hemi-depletion. No responses (<5 pg/ml background) were detected against irrelevant g280 peptide-loaded T2 cells or HLA-A2(-)ve MART-1(+)ve melanoma cells (TC938, TC888).

These observations demonstrate that TILs produced under perfusion culture conditions express high functionality and melanoma tumor antigen specificity. These results may reflect the high metabolic activity of T-cells harvested from perfusion cultures or other biological effects of perfusion in combination with the unique microenvironment and cellular interactions produced in high density cultures.

Clinical activity has been shown to correlate positively with the ability of T-cells (including Tumor infiltrating lymphocytes or "TILs") to produce GM-CSF (Cole et al., 1994;[1] Schwartzentruber et al.,[2] 1994; Ridolfi et al., 2003[3]). Note that GM-CSF is produced at high levels by T-cells derived under our examples (see attached Figures).

Further, the capacity for proliferation is widely believed to provide a key function for T-cell therapeutic efficacy. For example, long term differentiation and expansion of T-cells is associated with telomere shortening (Rufer et al., 1998;[4] Reed et al., 2004[5]) and proliferative "exhaustion" or senescence. Shortening of telomeres is associated with loss of T-cell function including proliferation (Blackburn, 2001;[6] de Lange, 2002[7]). Furthermore, Zhou et al. (2005)[8] report that T-cells demonstrating the greatest therapeutic efficacy against cancer had the greatest telomere lengths, highest proliferative potential and longest persistence *in vivo* in humans. In contrast, telomere shortening was associated with loss of

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[1] *Cancer Immunol Immunother 38*:299-303.
[2] *J Clin Oncol 12*:1475-1483.
[3] *J Immunother 26*:156-162.
[4] *Nat.Biotechnol.* 16:743-747.
[5] *J.Exp.Med.* 199:1433-1443.
[6] *Cell* 106:661-673.
[7] *Oncogene*. 21(4):532-40.
[8] *J.Immunol.* 175:7046-7052.

proliferative potential, poor persistence *in vivo* and lack of objective clinical responses in human patients as measured by tumor shrinkage and patients' survival.

Therefore, high proliferative potential and cytokine release at the time of harvest as demonstrated in the examples provided in the specification and the data presented here demonstrate their usefulness for the therapeutic efficacy of these T-cells after re-infusion into human patients.

In the bottom half of page 3 of the Office communication dated October 11, 2007 there is mention of the data disclosed in Table 1 of the specification. The rejection mentions the data disclosed in Table 1 of the specification comparing the proliferation potential between cells that have been cultured in T-flasks or perfusion bioreactor for 4 days.

To clarify this point as it relates the data presented in the specification, PHA-activated $CD8^+$ T-cells from the same inoculum source first were grown in separate side-by-side primary cultures for 10 days under either: 1) low density hemi-depletion conditions in T-flasks (standard methodology analogous to '358 and/or Stacey et al.), or 2) perfusion culture conditions using preferred conditions for the new methodology in the clinical-scale bioreactor. For these primary cultures, T-lymphocytes growing in T-flasks were counted daily and diluted to 0.5×10^6 T-cells/ml when density exceeded 1×10^6 T-cells/ml. In contrast, T-cells derived under perfusion conditions were expanded to high density ($12 - 32 \times 10^6$ T-cells/ml) in a continuous process without subculture while ramping medium perfusion to maintain a lactate concentration of 0.5 mg/ml as described in the application, examples and claims. Both sets of primary cultures were incubated for 10 days prior to harvest and transfer in parallel to secondary cultures. In fact, fold-expansion in the primary perfusion cultures is initially somewhat lower than conventional T-flasks (Table 1) most likely as a result of contact inhibition at very high cell density.

As shown in the specification to assess proliferative capability after harvest of each primary culture on day 10, the T-cells from each group (T-flask or perfusion bioreactor) separately were re-suspended in fresh medium containing T-cell growth factor (interleukin-2) and cultured under identical conditions at low density using hemi-depletion in T-flasks (Secondary cultures). Thus, each primary T-cell population was transferred separately to secondary T-flasks to provide identical conditions for secondary culture and evaluation of post-harvest proliferation potential.

II. The claims would not have been obvious based on what is described by US '358 and Stacey

Conventional wisdom based on '358 or Stacey et al, would predict that T-cells cultured at high density are contact inhibited at the time of harvest on day 10 and poorly proliferative in secondary cultures. In contrast, the proliferative potential of T-cells derived under the medium exchange conditions of the present application (high T-cell density in primary culture) very unexpectedly was greater than conventionally derived T-cells (low T-cell density in primary culture) as assessed in these secondary cultures. In fact, extension of the secondary culture duration to a total of 14-21 days did not alter the results or conclusions of this experiment as the T-cells derived under conventional hemi-depletion conditions had ceased proliferation by 4 days of secondary culture.

These experiments demonstrate that the post-harvest proliferation potential of T-cells derived in the Aastrom Cell Production System (CPS) under conditions with continuous medium perfusion is greater than the post-harvest proliferation potential of control T-cells derived in conventional T-flasks. Thus, the overall potential for T-cell expansion is significantly higher (3.8 – 10 fold) after growth under conditions of continuous medium perfusion when compared to conventional T-flasks.

As shown in the application and additional examples provided herein, T-cells cultured at high density under the medium exchange culture conditions (of the claims) exhibited enhanced cytokine release compared to control hemi-depletion methodology. These T-cells demonstrated enhanced cytokine secretion in response to triggering using polyclonal activators such as anti-CD3 monoclonal antibody or specific triggering using melanoma tumor antigens including peptide epitopes or endogenous tumor antigens expressed by a panel of patient-derived melanoma tumor cells.

These data demonstrate superior cytokine release extending beyond our original example of PHA-activated CD8+ T-cells to include clinically relevant TILs from a melanoma patient. In addition the experiment demonstrates tumor antigen specificity (i.e. the expanded TILs not only secrete higher levels of cytokine using our method but also respond only to tumor-antigen positive targets and not against irrelevant stimulating cells in a melanoma tumor cell panel).

Obtaining T-cells with enhanced proliferative potential and cytokine secretion under these continuous culture conditions is entirely unexpected and counter-intuitive to those skilled in the art as indicated above (i.e. conventional wisdom emphasizes splitting cultures to maintain low density).

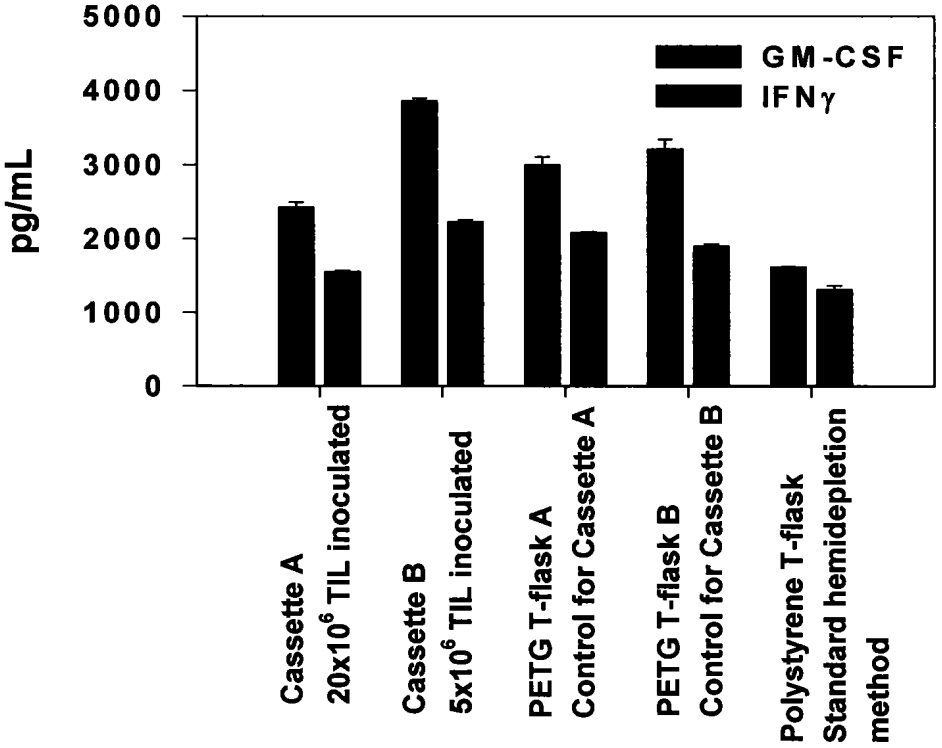
The undersigned declares further that all statements made herein are of his own knowledge are true and that all statements made on information are believed to be true. Further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Signature Dwight M. Smith

Date February 4, 2008

Figure 1. Functional activity and specificity of MART-1 reactive TILs produced under perfusion culture conditions in the clinical-scale bioreactor system, small-scale tissue culture flasks mimicking conditions used in the bioreactor system, or control hemi-depletion tissue culture flasks. A) Cytokine release (IFNgamma and GM-CSF) in response to immobilized anti-CD3 mAb; B) Cytokine release (IFNgamma) in response to peptide loaded T2 cells or a panel of melanoma cell lines. Cytokine release is expressed as concentration in picograms per ml as determined by ELISA.

A.



B.